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Article

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Green Synthesis of Potential Antifungal Agents: 2-Benzyl Substituted Thiobenzoazoles.

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ABSTRACT

A series of benzyl-substituted thiobenzoazoles were synthesized by an environmentally friendly approach, to search for new antifungal agrochemicals. Compounds were prepared starting from 2-mercaptobenzoazoles, using KOH, benzyl halides and water, resulting in a simple and ecological method. New antifungals were tested against a group of phytopathogenic fungi. Two compounds showed an interesting activity against *Botrytis cinerea*, *Fusarium oxysporum* and *Aspergillus* spp.: 2-((4-(trifluoromethyl)benzyl)thio)benzo[*d*]thiazole, **3ac**, and 2-((4-methylbenzyl)thio)benzo[*d*]thiazole, **3al**. Thus, **3ac** and **3al** can be considered as broad spectrum antifungal agents. Furthermore, two new compounds, 2-((4-iodobenzyl)thio)benzo[*d*]thiazole, **3aj,** and 2-(benzylthio)benzo[*d*]oxazole, **3ba**, showed better inhibitory effect against *Botrytis cinerea* and *Fusarium oxysporum* when compared to the commercial fungicide Captan. Thus, **3aj** and **3ba** can be considered reduced-spectrum antifungals.

KEYWORDS: *synthesis*, *antifungal activity*, *benzothiazole*, *benzoxazole*.

INTRODUCTION

Major crops are worldwide affected by several pests and diseases. In Argentina, as in other regions of the world, fungi are important agricultural pests. Both wheat (*Triticum* spp.) and maize (*Zea mays* L.) are among the most important crops in Argentina, either for local consumption or for export. These crops may be contaminated by a variety of toxigenic mold species, mainly by *Aspergillus*,[1] *Fusarium*,[2] *Penicillium*[3] and *Botrytis*,[4] causing health concerns and significant economic losses. *Botrytis cinerea* is a necrotrophic fungal pathogen that attacks over 200 different plant species, causing serious losses in the grape production and wine industry.[5,6] Fusariosis is a disease caused by several species of the genus *Fusarium*, which affects the major crops, such as wheat,[7] corn, soybean,[8,9] barley and other small grains, causing great economic losses. Micotoxins (aflatoxins,[10] ochratoxins,[11,12] among others) produced by *Aspergillus* spp. can cause economic and health problems since they contaminate foods and feeds for both humans and animals. Chemical protection against fungal disease is based on the use of protective fungicides. One of the most used fungicides is Captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) (Figure 1A). It has been classified as carcinogenic by the International Agency for Research on Cancer,[13] being also considered as sensitizing and strong irritant of eyes, skin and respiratory tract.[14–22] Such adverse effects trigger the need for designing new antifungals, more effective and less toxic than the current commercial compounds.

Some benzoazoles such as benzimidazole, benzothiazole and benzoxazole are often incorporated as building blocks in medicinal chemistry studies.[23] Benzothiazole derivatives find use in various branches of chemical research, for instance, in polymer chemistry, dyes, drugs, among others (Figure 1B).[23] The benzoxazole scaffold is a constituent of several natural products (Figure 1C),[24–26] and is often incorporated in drug design. Among the various biological applications reported for these heterocycles, the fungicidal activity[27–32] is recurrent. Thus, compounds derived from benzimidazole, benzothiazole and benzoxazole have been extensively used in the clinic, for

preventing and treating various types of diseases, showing low toxicity, high bioavailability, good biocompatibility and curative effects.[33]

Different synthetic methodologies to obtain 2-benzyl-substituted thiobenzoazoles have been reported. Among these synthetic strategies, it is worth to mention reactions between 2-mercaptobenzoazoles and benzyl halides. These reactions have different issues regarding green approaches, such as heterogeneous catalysts,[34] metalic sodium[35] or refluxing acetone[36] employment. Other alternatives imply reactions between 2-mercaptobenzoazoles and benzyl alcohols. For this route, most conditions imply usage of environmentally problematic or hazardous solvents[37] or reactants, such as $SOCl_2$,[38] $ClPPh_2$,[39] dioxane,[40] dichloromethane,[38,39] acetonitrile,[41] among others. Other synthetic strategies involve use of different benzyl sources, such dibenzyl carbonate,[42] oligomeric benzylsulfonium salts,[43] or toluene derivatives.[44] Also, there are alternatives for the benzoazole insertion, such as the microwave-assisted two-step reactions between *o*-halo-anilines, potassium *O*-ethyl dithiocarbonate (which form 2-mercptobenzothiazole as intermediate) and benzyl bromides,[45] or reaction between 3*H*-benzoazole-2-thione (easily interconvertible 2-mercaptobenzoazole isomer) and *O*-benzyl-isoureas.[40] Summarizing, some of these methodologies involve unsafe conditions or utilization of environmentally problematic or hazardous solvents. There are also cases where atom economy is compromised by adding the benzyl source in excess. It is as well remarkable that many of these methodologies require of previous preparation of substrates and transition metal or organo-catalysis employment. We have recently reported a new synthesis of 2-aryl thiobenzothiazoles using two methodologies (photostimulation or microwave assistance). These new antifungals were tested by the diffusion method against *B. cinerea*, showing an interesting activity.[28] Thus, the main goal of this work was to obtain 2-benzyl-substituted thiobenzothiazoles and thiobenzoxazoles by a simple and eco-friendly methodology, starting from readily available materials and using water[46] as solvent. Additionally, evaluation of the difference in the antifungal activity when changing the substituted benzyl groups in the new synthesized compounds was of interest. Thus, the activity of these new compounds against different

phytopathogenic fungi, such as *Botrytis cinerea*, *Fusarium oxysporum* and *Aspergillus spp*. was tested.

MATERIALS AND METHODS

Chemicals. 2-mercaptobenzothiazole, 2-mercaptobenzoxazole, benzyl halides, KOH, were commercially available (Sigma, St. Louis, MO) and used as received. Ultrapure water (MilliQ) was employed for all reactions.

Microorganisms. New compounds were assayed against: a) *Botrytis cinerea* isolated from *Vitis vinifera* cv. Chardonnay, cultivated in Province of San Juan, Argentina; b) *Aspergillus ustus* (PN-S4); c) *Aspergillus terreus* (M16C); d) *Fusarium oxysporum* (M15-Pa) (strains b-d were isolated from soil samples from the Province of San Juan, Argentina); e) *Aspergillus fumigatus* (ATTC 26934); g) *Aspergillus niger* (ATCC 9029). The microorganisms were grown in Czapek medium (Sigma, St. Louis, MO), enriched with a solution of mineral salts, at 30 °C for *Aspergillus* spp. and *Fusarium oxysporum*, and at 22 °C for *Botrytis cinerea*, for a period of 5 - 10 days.

Reactions of benzyl halides with 2-mercaptobenzoazoles. 2-mercaptobenzothiazole or 2-mercaptobenzoxazole (0.6 mmol), KOH (0.5 mmol) and benzyl halide (0.5 mmol) were vigorously mixed in 2 mL of ultrapure water in a sealed glass vessel, letting to react for 30 minutes at 50 °C. Afterwards, the reaction mixture was extracted three times with ethyl acetate (2 mL each). The combined organic layer was washed three times with ultrapure water (2 mL each), dried with anhydrous Na_2SO_4, analyzed by gas chromatography (GC). The product was purified by circular layer chromatography (CLC) using pentane/ethyl acetate (90:10) as mobile phase.

Antifungal susceptibility test. Antifungal activity was determined by applying the broth microdilution method, in accordance to Clinical and Laboratory Standard Institute (CLSI) (M27-A3 for yeasts and M38-A2 for molds). Assays were performed in enriched Czapek broth. The inoculum employed was 1 - 5 × 10^5 conidia/mL. Stock solutions of tested compounds were prepared in DMSO to give serial two-fold dilutions to final concentrations of 3.1 - 100 mg/L (final DMSO

concentration ≤2%). Microtiter plates were incubated at 30 ºC for *Aspergillus* and *Fusarium* spp., and at 22 ºC for *Botrytis cinerea* in a moist and dark chamber. Inhibitory concentrations were recorded after 48 h for *Aspergillus* and *Fusarium* spp. and after 72 h for *B. cinerea*, according to the control fungal growth. A positive control of the commercial antifungal Captan and solvent control using 2% aqueous DMSO were included. All tests were run in triplicate. The 50% Inhibitory concentration (IC_{50}) was defined as the minimum concentration of the compound that resulted in 50% inhibition of the fungal growth. IC_{50} values were calculated by linear regression plots of %(inhibition) *vs* ln(concentration), considering 50% inhibition.

2-(benzylthio)benzo[*d*]thiazole, 3aa:[35] Yellow solid, mp: 37.8 - 38.7 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.60 (s, 2H), 7.24 - 7.34 (m, 4H), 7.40-7.46 (m, 3H), 7.74 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 8.1 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 37.73, 121.02, 121.59, 124.30, 126.08, 127.77, 128.73, 129.16, 135.36, 136.21, 153.19, 166.42. MS (EI^+) *m/z* (%): 259.20 (9), 258.20 (14), **257.15** (79), 224.00 (72), 165.95 (8), 107.90 (13), 91.00 (100), 89.10 (5), 65.05 (33), 63.00 (9). HRMS (ESI): (M+Na): $C_{14}H_{11}NS_2Na$, calculated: 280.0225, found: 280.0233.

2-((4-fluorobenzyl)thio)benzo[*d*]thiazole, 3ab: Yellow solid, mp: 66.3 - 67.0 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.57 (s, 2H), 7.00 (tt, J = 8.7 Hz, J = 2.1 Hz, 2H), 7.30 (ddd, J = 8.1 Hz, J = 7.3 Hz, J = 1.2 Hz, 1H), 7.40 - 7.44 (m, 3H), 7.74 (ddd, J = 7.9 Hz, J = 1.1 Hz, J = 0.5 Hz, 1H), 7.89 (ddd, J = 8.1 Hz, J = 1.0 Hz, J = 0.5 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 36.88, 115.60 (d, $J^2_{F\text{-}C}$ = 21.68 Hz), 121.04, 121.60, 124.39, 126.11, 130.81 (d, $J^3_{F\text{-}C}$ = 8.2 Hz), 132.16 (d, $J^4_{F\text{-}C}$ = 3.2 Hz), 135.38, 153.12, 162.30 (d, $J^1_{F\text{-}C}$ = 246.5 Hz), 165.99. ^{19}F-NMR (376.5 MHz, $CDCl_3$), δ (ppm): -144.33 (tt, $J^o_{F\text{-}H}$ = 8.7 Hz, $J^m_{F\text{-}H}$ = 4.5 Hz). MS (EI^+) *m/z* (%): 276.20 (5), **275.15** (32), 242.10 (22), 166.00 (3), 109.10 (100), 107.80 (8), 89.10 (1), 83.10 (13), 63.05 (4). HRMS (ESI): (M+Na): $C_{14}H_{10}NS_2FNa$, calculated: 298.0131, found: 298.0138.

2-((4-(trifluoromethyl)benzyl)thio)benzo[*d*]thiazole, 3ac: Yellow solid, mp: 87.8 - 88.3 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.64 (s, 2H), 7.31 (td, J = 7.6 Hz, J = 1.1 Hz, 1H), 7.43 (td, J = 7.7 Hz, J = 1.2 Hz, 1H), 7.58 (s, 4H), 7.75 (dd, J = 8.0 Hz, J = 0.5 Hz, 1H), 7.90 (dd, J = 8.1 Hz, J

= 0.5 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 36.84, 121.08, 121.64, 124.03 (q, J^1_{F-C} = 272.2 Hz), 124.49, 125.62 (q, J^3_{F-C} = 3.7 Hz), 126.16, 129.46 (q, J^4_{F-C} = 0.5 Hz), 129.92 (q, J^2_{F-C} = 32.5 Hz), 135.44, 140.80 (q, J^5_{F-C} = 0.8 Hz), 153.04, 165.41. ^{19}F-NMR (376.5 MHz, $CDCl_3$), δ (ppm): -62.58 (s). MS (EI^+) *m/z* (%): 327.20 (9), 326.25 (14), **325.10** (95), 292.10 (66), 223.10 (17), 180.10 (10), 165.95 (21), 159.05 (100), 133.05 (3), 121.90 (14), 119.05 (11), 109.10 (47), 107.95 (29), 89.05 (7), 63.05 (10). HRMS (ESI): (M+Na): $C_{15}H_{10}NS_2F_3Na$, calculated: 348.0099, found: 348.0109.

4-((benzo[*d*]thiazol-2-ylthio)methyl)benzonitrile, 3ad:[35] Yellow solid, mp: 64.0 - 65.0 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.62 (s, 2H), 7.31 (td, *J* = 7.6 Hz, *J* = 1.1 Hz, 1H), 7.43 (td, *J* = 7.7 Hz, 1.2 Hz, 1H), 7.56 - 7.61 (m, 4H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 8.1, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 36.83, 111.55, 118.58, 121.11, 121.66, 124.60, 126.21, 129.86, 132.41, 135.48, 142.44, 152.94, 164.94. MS (EI^+) *m/z* (%): 284.20 (12), 283.25 (19), **282.15** (99), 250.25 (18), 249.05 (100), 179.95 (15), 166.00 (26), 122.00 (16), 116.10 (85), 108.00 (31), 90.10 (10), 89.00 (37), 76.05 (3), 63.05 (13). HRMS (ESI): (M+Na): $C_{15}H_{10}N_2S_2Na$, calculated: 305.0178, found: 305.0180.

2-((4-nitrobenzyl)thio)benzo[*d*]thiazole, 3ae:[35] Yellow solid, mp: 89.7 - 91.4 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.66 (s, 2H), 7.31 (td, *J* = 7.6 Hz, *J* = 0.8 Hz, 1H), 7.43 (td, *J* = 7.7 Hz, *J* = 0.9 Hz, 1H), 7.63 (d, *J*= 8.6 Hz, 2H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 8.16 (d, *J* = 8.7 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 36.47, 121.13, 121.67, 123.84, 124.63, 126.24, 130.00, 135.48, 144.54, 147.36, 152.91, 164.77. MS (EI^+) *m/z* (%): 304.20 (12), 303.25 (16), **302.15** (100), 269.10 (21), 223.10 (50), 179.95 (13), 165.95 (22), 136.10 (9), 122.10 (13), 110.10 (2), 108.00 (28), 106.05 (18), 90.10 (29), 89.05 (29), 78.10 (36), 63.05 (14). HRMS (ESI): (M+Na): $C_{14}H_{10}N_2S_2O_2Na$, calculated: 325.0076, found: 325.0089.

2-((2-nitrobenzyl)thio)benzo[*d*]thiazole, 3af:[35] Brown solid, mp: 67.1 - 68.5 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.95 (s, 2H), 7.28 (td, *J* = 7.6 Hz, *J* = 1.0 Hz, 1H), 7.39 - 7.44 (m, 2H), 7.54 (td, *J* = 7.6 Hz, *J* = 1.2 Hz, 1H), 7.7 (d, *J* = 7.7 Hz, 1H), 7.81 (dd, *J* = 7.7 Hz, *J* = 1.0 Hz, 1H), 7.90

(d, J = 8.1 Hz, 1H), 8.05 (dd, J = 8.2 Hz, J = 1.0 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 34.20, 121.05, 121.59, 124.39, 125.24, 126.07, 128.72, 132.75, 133.35, 133.52, 135.57, 148.37, 152.92, 165.67. MS (EI^+) *m/z* (%): 303.25 (2), **302.20** (11), 223.10 (12), 168.10 (12), 167.00 (100), 165.95 (5), 136.15 (15), 122.10 (8), 110.10 (1), 108.10 (18), 106.15 (4), 89.10 (11), 78.10 (41), 77.10 (11), 65.10 (13), 63.05 (10). HRMS (ESI): (M+Na): $C_{14}H_{10}N_2S_2O_2Na$, calculated: 325.0076, found: 325.0084.

2-((2-chlorobenzyl)thio)benzo[*d*]thiazole, 3ag: Yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.73 (s, 2H), 7.17 - 7.24 (m, 2H), 7.29 (td, J = 7.7 Hz, J = 1.1 Hz, 1H), 7.38 - 7.44 (m, 2H), 7.59 (dd, J = 6.9 Hz, J = 2.3 Hz, 1H), 7.74 (dd, J = 7.9 Hz, J = 0.5 Hz, 1H), 7.91 (dd, J = 8.1 Hz, J = 0.4 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 35.26, 121.03, 121.60, 124.33, 126.05, 127.01, 129.19, 129.74, 131.30, 134.40, 134.44, 135.50, 153.14, 166.08. MS (EI^+) *m/z* (%): 292.80 (24), **291.00** (44), 257.75 (30), 255.95 (83), 223.05 (31), 165.85 (8), 126.85 (43), 124.95 (100), 122.05 (6), 107.85 (15), 98.95 (10), 90.05 (8), 89.00 (29), 62.95 (14). HRMS (ESI): (M+Na): $C_{14}H_{10}NS_2ClNa$, calculated: 313.9835, found: 313.9845.

2-((4-bromobenzyl)thio)benzo[*d*]thiazole, 3ah:[36] Yellow solid, mp: 80.3 - 81.0 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.54 (s, 2H), 7.28 - 7.34 (m, 3H), 7.40 - 7.45 (m, 3H), 7.74 (dd, J = 8.0 Hz, J = 0.5 Hz, 1H), 7.89 (dd, J = 8.2 Hz, J = 0.4 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 36.92, 121.05, 121.61, 121.71, 124.42, 126.13, 130.82, 131.81, 135.40, 135.56, 153.08, 165.74. MS (EI^+) *m/z* (%): 337.05 (70), **335.10** (60), 304.00 (20), 302.10 (17), 223.15 (53), 171.00 (84), 169.05 (100), 166.00 (19), 143.00 (1), 122.10 (12), 108.00 (24), 90.10 (52), 89.05 (40), 63.05 (18). HRMS (ESI): (M+Na): $C_{14}H_{10}NS_2BrNa$, calculated: 357.9330, found: 357.9346.

2-((2-bromobenzyl)thio)benzo[*d*]thiazole, 3ai:[36] Yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.74 (s, 2H), 7.13 (td, J = 7.7 Hz, J = 1.7 Hz, 1H), 7.24 (td, J = 7.6 Hz, J = 1.2 Hz, 1H), 7.29 (td, J = 7.1 Hz, J = 1.1 Hz, 1H), 7.43 (ddd, J = 8.3 Hz, J = 7.3 Hz, J = 1.2 Hz, 1H), 7.58 (dd, J = 8.0 Hz, J = 1.1 Hz, 1H), 7.61 (dd, J = 7.7 Hz, J = 1.6 Hz, 1H), 7.74 (dd, J = 8.0 Hz, J = 0.5 Hz, 1H), 7.91 (dd, J = 7.6 Hz, J = 0.4 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 37.91, 121.04,

121.59, 124.32, 124.83, 126.05, 127.66, 129.38, 131.36, 133.05, 135.51, 136.12, 153.13, 166.03. MS (EI^+) *m/z* (%): 336.90 (27), **334.95** (28), 301.80 (2), 257.10 (18), 256.05 (100), 224.25 (15), 223.05 (79), 170.90 (61), 168.95 (81), 166.00 (27), 122.05 (21), 108.05 (36), 90.05 (75), 89.05 (57), 63.00 (31). HRMS (ESI): (M+Na): $C_{14}H_{10}NS_2BrNa$, calculated: 357.9330, found: 357.9341.

2-((4-iodobenzyl)thio)benzo[*d*]thiazole, 3aj:[36] Yellow solid, mp: 68.3 - 69.8 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.52 (s, 2H), 7.20 (d, *J* = 8.3 Hz, 2H), 7.29 (td, *J* = 7.6 Hz, *J* = 1.1 Hz, 1H), 7.42 (td, *J* = 7.7 Hz, *J* = 1.1 Hz, 1H), 7.63 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 8.1 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 37.01, 93.25, 121.05, 121.61, 124.42, 126.12, 131.05, 135.40, 136.24, 137.78, 153.08, 165.74. MS (EI^+) *m/z* (%): 384.15 (9), **383.05** (75), 350.05 (14), 223.05 (34), 217.00 (100), 165.95 (19), 122.05 (12), 108.00 (22), 90.05 (58), 89.05 (45), 63.00 (18). HRMS (ESI): (M+Na): $C_{14}H_{10}NS_2INa$, calculated: 405.9192, found: 405.9202.

2-((2-iodobenzyl)thio)benzo[*d*]thiazole, 3ak: Yellow solid, mp: 48.2 - 49.4 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.72 (s, 2H), 6.95 (td, *J* = 7.7 Hz, *J* = 1.5 Hz, 1H), 7.35 - 7.21 (m, 2H), 7.42 (td, *J* = 8.3 Hz, *J* = 1.1 Hz, 1H), 7.60 (dd, *J* = 7.6 Hz, *J* = 1.4 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.85 (dd, *J* = 7.9 Hz, *J* = 0.8 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 42.78, 100.75, 121.04, 121.61, 124.33, 126.06, 128.55, 129.43, 130.68, 135.53, 139.30, 139.76, 153.14, 165.88. MS (EI^+) *m/z* (%): **383.05** (35), 258.15 (11), 257.20 (18), 256.10 (100), 224.20 (19), 223.10 (92), 217.00 (84), 165.90 (10), 122.10 (11), 121.10 (16), 108.05 (18), 90.15 (98), 89.10 (48), 63.05 (20). HRMS (ESI): (M+Na): $C_{14}H_{10}NS_2INa$, calculated: 405.9192, found: 405.9196.

2-((4-methylbenzyl)thio)benzo[*d*]thiazole, 3al:[41] Yellow solid, mp: 49.5 - 50.7 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 2.32 (s, 3H), 4.57 (s, 2H), 7.13 (d, *J* = 7.8 Hz, 2H), 7.28 (td, *J* = 7.6 Hz, *J* = 0.8 Hz, 1H), 7.33 (d, *J* = 7.9 Hz, 2H), 7.42 (td, *J* = 7.7 Hz, *J* = 4.6 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 21.16, 37.57, 121.00, 121.56, 124.25, 126.05, 129.06, 129.42, 133.03, 135.34, 137.57, 153.22, 166.62. MS (EI^+) *m/z* (%): 272.20 (8), **271.10** (48), 238.20 (28), 223.05 (3), 165.95 (5), 122.10 (3), 107.95 (6), 106.15 (12), 105.05

(100), 79.10 (15), 77.00 (17), 65.10 (2), 63.05 (3). HRMS (ESI): (M+Na): $C_{15}H_{13}NS_2Na$, calculated: 294.0382, found: 294.0380.

2-(benzylthio)benzo[*d*]oxazole, 3ba:[35] White solid, mp: 48.8 - 50.2 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.57 (s, 2H), 7.22 - 7.36 (m, 5H), 7.42 - 7.46 (m, 3H), 7.62 (ddd, J = 7.7 Hz, J = 1.5 Hz, J = 0.8 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 36.60, 109.91, 118.50, 123.95, 124.32, 127.92, 128.78, 129.09, 135.87, 141.95, 151.91, 164.47. MS (EI^+) *m/z* (%): 242.25 (18), **241.20** (74), 208.10 (34), 150.10 (6), 122.10 (20), 92.15 (28), 91.00 (100), 89.10 (8), 65.15 (40), 63.10 (16), 51.05 (11). HRMS (ESI): (M+Na): $C_{14}H_{11}NOSNa$, calculated: 264.0454, found: 264.0458.

2-((4-fluorobenzyl)thio)benzo[*d*]oxazole, 3bb: White solid, mp: 37.5 - 38.0 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.57 (s, 2H), 7.01 (tt, J = 8,6 Hz, J = 2.4 Hz, 2H), 7.24 (td, J = 7.7 Hz, J = 1.4 Hz, 1H), 7.29 (td, J = 7.6 Hz, J = 1.3 Hz, 1H), 7.42 - 7.45 (m, 3H), 7.62 (dd, J = 7.4 Hz, J = 1.1 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 35.80, 109.93, 115.68 (d, $J^2_{F\text{-}C}$ = 21.6 Hz), 118.52, 124.03, 124.36, 130.78 (d, $J^3_{F\text{-}C}$ = 8.3 Hz), 131.83 (d, $J^4_{F\text{-}C}$ = 3.1 Hz), 141.87, 151.92, 162.40 (d, $J^1_{F\text{-}C}$ = 246.8 Hz), 164.25. ^{19}F-NMR (376.5 MHz, $CDCl_3$), δ (ppm): -114.07 (tt, $J^o_{F\text{-}H}$ = 7.9 Hz, $J^m_{F\text{-}H}$ = 4.4 Hz). MS (EI^+) *m/z* (%): 260.25 (13), **259.25** (65), 226.20 (23), 150.10 (6), 122.10 (19), 110.15 (26), 109.10 (100), 107.15 (10), 89.15 (4), 83.05 (35), 63.10 (14). HRMS (ESI): (M+Na): $C_{14}H_{10}NOSFNa$, calculated: 282.0359, found: 282.0357.

2-((4-(trifluoromethyl)benzyl)thio)benzo[*d*]oxazole, 3bc: White solid, mp: 55.7 - 57.3 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.58 (s, 2H), 7.25 (td, J = 7.5 Hz, J = 1.5 Hz, 1H), 7.30 (td, J = 7.7 Hz, J = 1.4 Hz, 1H), 7.44 (dd, J = 7.5 Hz, J = 1.0 Hz, 1H), 7.59 (s, 4H), 7.62 (dd, J = 7.5 Hz, J = 1.1 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 35.81, 109.98, 118.57, 123.99 (q, $J^1_{F\text{-}C}$ = 272.1 Hz), 124.14, 124.43, 125.69 (q, $J^3_{F\text{-}C}$ = 3.7 Hz), 129.41, 130.12 (q, $J^2_{F\text{-}C}$ = 32.7 Hz), 140.37, 141.79, 152.00, 163.81. ^{19}F-NMR (376.5 MHz, $CDCl_3$), δ (ppm): -62.67 (s). MS (EI^+) *m/z* (%): 310.30 (12), **309.20** (77), 276.20 (37), 160.15 (11), 159.05 (100), 150.05 (18), 133.15 (1), 132.10

(5), 122.00 (33), 119.05 (8), 109.05 (31), 92.05 (1), 89.05 (5), 63.05 (10). HRMS (ESI): (M+Na): $C_{15}H_{10}NOSF_3Na$, calculated: 332.0327, found: 332.0330.

4-((benzo[*d*]oxazol-2-ylthio)methyl)benzonitrile, 3bd:[35] Orange solid, mp: 104.9 - 106.4 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.56 (s, 2H), 7.24 - 7.32 (m, 2H), 7.44 (dd, J = 7.4 Hz, J = 1.1 Hz, 1H), 7.58 - 7.63 (m, 5H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 35.83, 110.01, 111.77, 118.52, 118.60, 124.24, 124.49, 129.80, 132.49, 141.70, 141.96, 152.04, 163.48. MS (EI^+) *m/z* (%): 267.30 (20), **266.15** (99), 234.25 (10), 233.15 (64), 164.10 (2), 150.05 (56), 122.00 (50), 117.15 (17), 116.10 (100), 92.05 (2), 90.05 (8), 89.00 (38), 76.00 (3), 63.05 (19). HRMS (ESI): (M+Na): $C_{15}H_{10}N_2OSNa$, calculated: 289.0406, found: 289.0405.

2-((4-nitrobenzyl)thio)benzo[*d*]oxazole, 3be:[35] Yellow solid, mp: 105.3 - 106.6 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.60 (s, 2H), 7.24 - 7.32 (m, 2H), 7.44 (dt, J = 7.7 Hz, J = 0.8 Hz, 1H), 7.61 (dt, J = 7.5 Hz, J = 0.9 Hz, 1H), 7.66 (d, J = 8.7 Hz, 2H), 8.18 (d, J = 8.7 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 35.49, 110.03, 118.62, 123.92, 124.28, 124.52, 129.96, 141.67, 144.02, 147.49, 152.05, 163.34. MS (EI^+) *m/z* (%): 287.25 (15), **286.20** (100), 269.20 (2), 253.20 (24), 207.20 (17), 179.15 (1), 150.10 (64), 136.15 (31), 122.15 (77), 106.10 (29), 92.10 (2), 90.15 (28), 89.10 (38), 78.10 (53), 64.10 (13), 63.10 (25), 51.10 (11). HRMS (ESI): (M+Na): $C_{14}H_{10}N_2O_3SNa$, calculated: 309.0304, found: 309.0305.

2-((2-nitrobenzyl)thio)benzo[*d*]oxazole, 3bf:[35] Green solid, mp: 65.7 - 67.2 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.88 (s, 2H), 7.21 - 7.30 (m, 2H), 7.41 - 7.47 (m, 2H), 7.56 - 7.62 (m, 2H), 7.86 (d, J = 7.6 Hz, 1H), 8.09 (d, J = 8.1 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 33.79, 109.98, 118.51, 124.01, 124.35, 125.42, 129.05, 132.75, 133.11, 133.77, 141.76, 148.04, 152.10, 164.39. MS (EI^+) *m/z* (%): 287.15 (4), **286.20** (22), 240.20 (17), 223.30 (3), 221.20 (14), 167,15 (1), 152.15 (15), 151.10 (95), 150.10 (18), 136.15 (87), 122.10 (41), 106.15 (12), 92.10 (12), 91.10 (11), 89.10 (11), 78.10 (100), 77.05 (19), 65.10 (16), 64.05 (13), 63.05 (20), 51.05 (14). HRMS (ESI): (M+Na): $C_{14}H_{10}N_2O_3SNa$, calculated: 309.0304, found: 309.0302.

2-((2-chlorobenzyl)thio)benzo[*d*]oxazole, 3bg: White solid, mp: 62.9 - 64.2 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.67 (s, 2H), 7.19 - 7.26 (m, 3H), 7.29 (td, *J* = 7.7 Hz, *J* = 1.3 Hz, 1H), 7.40 (dd, *J* = 7.4 Hz, *J* = 1.9 Hz, 1H), 7.43 (dq, *J* = 8.0 Hz, *J* = 0.7 Hz, 1H), 7.60 - 7.64 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 34.32, 109.93, 118.51, 123.96, 124.31, 127.06, 129.41, 129.78, 131.27, 134.07, 134.45, 141.93, 152.01, 164.43. MS (EI^+) *m/z* (%): 277.10 (10), **275.05** (23), 242.15 (16), 241.20 (14), 240.05 (100), 207.10 (4), 150.00 (8), 127.05 (54), 126.10 (15), 125.00 (91), 121.95 (29), 99.00 (12), 92.05 (1), 90.05 (10), 89.05 (38), 63.00 (22). HRMS (ESI): (M+Na): $C_{14}H_{10}NOSClNa$, calculated: 298.0064, found: 298.0059.

2-((4-bromobenzyl)thio)benzo[*d*]oxazole, 3bh: Yellow solid, mp: 67.4 - 67.9 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.49 (s, 2H), 7.24 (td, *J* = 7.6 Hz, *J* = 3.2 Hz, 1H), 7.29 (td, *J* = 7.7 Hz, *J* = 1.4 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 7.42 - 7.46 (m, 3H), 7.61 (dt, *J* = 7.6 Hz, *J* = 0.8 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 35.84, 109.94, 118.54, 121.92, 124.06, 124.38, 130.75, 131.88, 135.20, 141.84, 151.95, 164.05. MS (EI^+) *m/z* (%): 321.00 (62), **319.00** (55), 288.00 (15), 286.10 (14), 207.15 (12), 170.95 (99), 168.95 (100), 150.00 (9), 142.95 (1), 121.90 (34), 92.05 (2), 90.05 (53), 89.05 (45), 63.05 (24). HRMS (ESI): (M+Na): $C_{14}H_{10}NOSBrNa$, calculated: 341.9559, found: 341.9565.

2-((2-bromobenzyl)thio)benzo[*d*]oxazole, 3bi: Yellow solid, mp: 68.2 - 69.3 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.68 (s, 2H), 7.15 (td, *J* = 7.7 Hz, *J* = 1.6 Hz, 1H), 7.22 - 7.31 (m, 3H), 7.43 (dd, *J* = 7.5 Hz, *J* = 0.8 Hz, 1H), 7.59 (dd, *J* = 8.0 Hz, *J* = 0.9 Hz, 1H), 7.62 - 7.65 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 36.99, 109.94, 118.50, 123.96, 124.32, 124.81, 127.72, 129.60, 131.35, 133.10, 135.78, 141.92, 152.00, 164.39. MS (EI^+) *m/z* (%): 320.95 (13), **319.05** (11), 241.20 (17), 240.10 (100), 207.15 (11), 170.95 (68), 168.95 (72), 150.00 (7), 122.05 (25), 92.05 (1), 90.10 (41), 89.05 (35), 63.00 (20). HRMS (ESI): (M+Na): $C_{14}H_{10}NOSBrNa$, calculated: 341.9559, found: 341.9567.

2-((4-iodobenzyl)thio)benzo[*d*]oxazole, 3bj: Brown solid, mp: 83.6 - 85.3 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.48 (s, 2H), 7.21 (d, *J* = 8.3 Hz, 2H), 7.25 (dd, J = 7.7 Hz, *J* = 1.4 Hz, 1H),

7.29 (td, J = 7.4 Hz, J = 1.1 Hz, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.61 (d, J = 7.4 Hz, 1H), 7.65 (d, J = 8.3 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 35.95, 93.46, 109.94, 118.54, 124.06, 124.38, 130.97, 135.87, 137.86, 141.84, 151.95, 164.05. MS (EI^+) *m/z* (%): **367.25** (79), 334.10 (16), 217.00 (100), 207.10 (8), 150.00 (4), 122.05 (14), 90.05 (52), 89.00 (39), 63.00 (16). HRMS (ESI): (M+Na): $C_{14}H_{10}NOSINa$, calculated: 389.9420, found: 389.9424.

2-((2-iodobenzyl)thio)benzo[*d*]oxazole, 3bk: Brown solid, mp: 69.0 - 70.3 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 4.67 (s, 2H), 6.97 (td, J = 7.7 Hz, J = 1.5 Hz, 1H), 7.36 - 7.17 (m, 3H), 7.43 (dd, J = 7.2 Hz, J = 0.7 Hz, 1H), 7.62 - 7.66 (m, 2H), 7.86 (dd, J = 7.9 Hz, J = 0.9 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 41.83, 100.65, 109.94, 118.52, 123.96, 124.32, 128.60, 129.62, 130.68, 138.96, 139.80, 141.94, 152.01, 164.23. MS (EI^+) *m/z* (%): **367.20** (8), 241.20 (16), 240.10 (100), 217.00 (91), 207.10 (8), 15.05 (3), 122.10 (11), 90.15 (58), 89.10 (32), 63.05 (13). HRMS (ESI): (M+Na): $C_{14}H_{10}NOSINa$, calculated: 389.9420, found: 389.9435.

2-((4-methylbenzyl)thio)benzo[*d*]oxazole, 3bl: White solid, mp: 56.1 - 57.9 °C. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 2.33 (s, 3H), 4.53 (s, 2H), 7.14 (d, J = 7.8 Hz, 2H), 7.31 - 7.19 (m, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.43 (dd, J = 7.5 Hz, J = 0.9 Hz, 1H), 7.61 (d, J = 7.5 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 21.13, 36.42, 109.88, 118.48, 123.90, 124.28, 129.00, 129.46, 132.73, 137.74, 141.99, 151.89, 164.67. MS (EI^+) *m/z* (%): 256.20 (6), **254.95** (47), 221.90 (11), 150.05 (2), 122.00 (6), 106.15 (16), 105.00 (100), 103.05 (10), 89.00 (1), 79.10 (15), 77.00 (18), 65.05 (2), 63.05 (4). HRMS (ESI): (M+Na): $C_{15}H_{13}NOSNa$, calculated: 278.0610, found: 278.0608.

RESULTS AND DISCUSSION

In order to synthesize different compounds with antifungal activity, reaction conditions were optimized for the nucleophilic substitution of benzyl halides, using 2-mercaptobenzothiazole (**1a**) and benzyl chloride (**2a**) as representative substrates. Water was chosen as solvent, as it is low cost, abundant and safe, so reducing negative environmental effects.

In a first instance, reaction between **1a** and **2a** was carried out using 2 equivalents of KOH to ensure full deprotonation of the thiol group. Reaction conditions were 100 °C for 24 hours, ensuring 99% substrate conversion with the concomitant product formation.

Afterwards, reaction time, temperature and base concentration were adjusted. Optimized reaction conditions were 30 minutes, 50 °C and one equivalent of KOH, reaching a conversion of 97%. In absence of base, only 51% conversion was reached, which is in accordance to a p*Ka* of *ca*. 7.65 reported for 2-mercaptobenzothiazole,[47] indicating an increment in the reaction rate as the formed anion fraction increases. Thus, to verify this behavior, one equivalent of different bases, such as NaOH, K_2CO_3, $NaHCO_3$ or sodium acetate were tested. Results of these last reactions did not show significant differences in conversion percentages, demonstrating a general basic catalysis. The method scope was further evaluated for 2-mercaptobenzothiazole (**1a**) or 2-mercaptobenzoxazole (**1b**) and a series of benzyl halides (Table 1) using the previously optimized conditions (1 equivalent of KOH, 50 °C, 30 minutes). Substrates substituted with electron withdrawing (Table 1, entries 2 to 11) and electron donating groups (Table 1, entry 12) were tested, without observing differences in mesomeric or inductive effects on the reactivity. Moreover, no steric effects were observed, as it can be seen from conversions from *ortho*- (Table 1, entry 6) and *para*-substituted substrates (Table 1, entry 5). It is worth noting the versatility of this synthetic method for benzyl thioethers, affording conversions from 91- 99%, without by-products formation.

Growth inhibition assay of phytopathogenic fungi.

Fungal growth was evaluated at six concentrations (3.1 - 100 mg/L), using enriched Czapek broth in the presence of compounds **3aa - 3bl**. Each treatment was carried out in triplicate. The 50% Inhibitory Concentrations (IC_{50}) are summarized in Table 2.

New antifungal compounds showed selectivity against some of the assayed microorganisms. The best effect against *B. cinerea* was observed for the compounds **3ac**, **3ad**, **3ag**, **3ah**, **3aj**, **3al**, **3bb**, **3bg**, **3bi** and **3bl**, showing IC_{50} values significantly equal or lower than the commercial compound (**3ag**, 1.1 μM; **3aj**, 1.4 μM; **3bi**, 1.4 μM; Captan, 17.8 μM). Comparing results obtained for **3aj** and

3ag with their aryl analogues 2-(4-iodophenylthio)benzo[*d*]thiazole and 2-(2-chlorophenylthio)benzo[*d*]thiazole, presented in our previous report,[28] (IC_{50} 2.63 μM and 2.5 μM, respectively), it could be suggested that the bridge expansion between benzothiazole and aryl groups, in these structures in particular, could positively influence the biological activity against *B. cinerea.*

Several tested compounds (**3aa**, **3ab**, **3ac**, **3ad**, **3ae**, **3ag**, **3ak**, **3al**, **3bc**, **3bd**, **3be**, **3bf**, **3bk** and **3bl**) showed significant activity against different species of *Aspergillus* spp. The best activity against *A. fumigatus* was confirmed for the compound **3bc**, with an IC_{50} of 5 μM, higher than the positive control (Captan, 15 μM). On the other hand, compounds **3ab** and **3aa** had the highest activity against *A. niger* (6.5×10^{-39} μM and 5 μM, respectively), even higher than Captan (24 μM). In addition, compound **3aa** also showed an interesting activity against *A. terreus,* with an IC_{50} of 13.2 μM, in comparison with an IC_{50} of 55.8 μM for Captan. Moreover, a greater effect was observed against *A. ustus* by the compound **3ac** (0.0119 μM) compared to Captan (61 μM).

Most compounds (**3aa**, **3ab**, **3ac**, **3ad**, **3ae**, **3af**, **3ag**, **3ah**, **3ak**, **3al**, **3ba**, **3bb**, **3bc**, **3bf**, **3bg**, **3bh** and **3bi)** had an activity similar or better than Captan (49 μM) against *F. oxysporum*. Furthermore, compounds **3aa** (0.23 μM), **3ab** (0.0067 μM) and **3ba** (3.0 μM) showed outstanding activity against this mold. In general, most of the synthesized compounds showed an important antifungal activity against the assayed fungi. No evident differences were observed when comparing benzothiazole and benzoxazole heterocycles. Moreover, the influence of electron withdrawing or donating groups in the benzyl moiety was also not evident.

Among the derivatives tested, compounds **3ag**, **3aj**, and **3bi** had remarkable growth inhibition for *Botrytis cinerea*. Compounds **3aa**, **3ab** and **3ba** were outstanding inhibitors of *Fusarium oxysporum*. In addition, compounds **3aa**, **3ab**, **3ac** and **3bc** showed an interesting activity against *Aspergillus* spp. Compounds **3ac** and **3al** can be highlighted as potential high-spectrum antifungals. Moreover, it could be suggested that having an extra carbon atom in the *para* position of the benzyl substituent would be useful for a better interaction within the target active site. Additionally,

363 compounds **3aj** and **3ba** could be considered as reduced-spectrum antifungals, since they showed
364 specificity against *B. cinerea* and *F. oxysporum*, respectively. These results suggest that these
365 compounds could be a valuable alternative to the antifungal agents currently used.
366 Summarizing, a practical and environmentally friendly method has been developed for a facile and
367 green synthesis of benzyl substituted thiobenzoazoles, starting from 2-mercaptobenzoxazole or 2-
368 mercaptobenzothiazole and benzyl halides, affording excellent yields. Most reported derivatives
369 proved to be better inhibitors than the commercial antifungal Captan, which has undesirable effects
370 on both health and environment.
371

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375

376 SUPPORTING INFORMATION DESCRIPTION

377 Reaction optimization, more details about antifungal susceptibility tests, spectroscopy data for all
378 compounds. This material is available free of charge via the Internet at http://pubs.acs.org.
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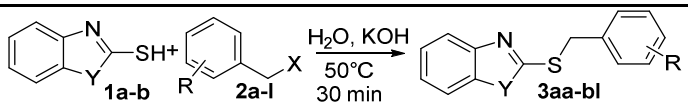
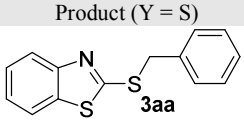
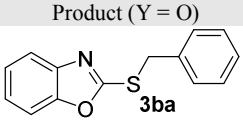
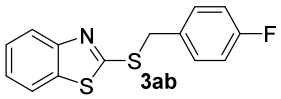
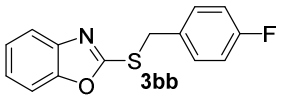
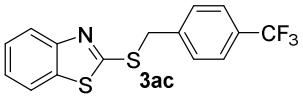
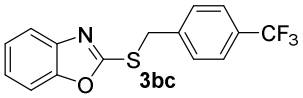
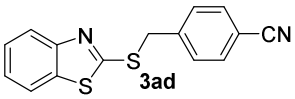
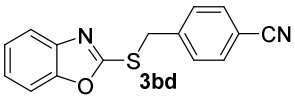
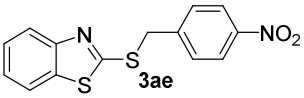
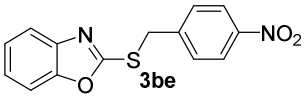
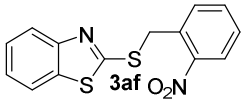
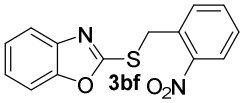
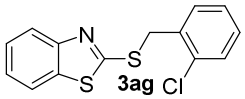
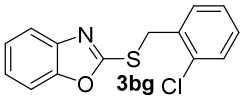
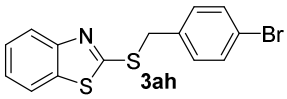
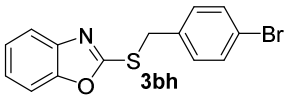
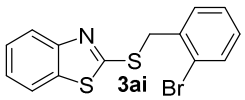
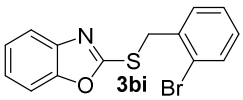
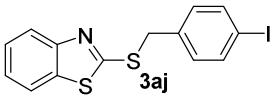
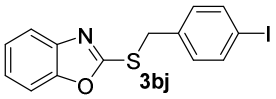
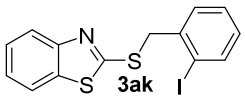
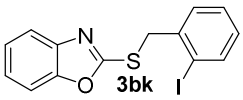
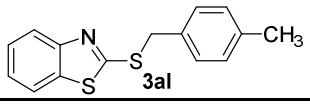
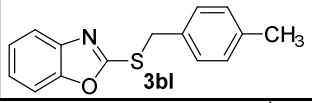
FIGURE CAPTIONS

Figure 1. (A) Captan, positive control. (B) Benzothiazole (red), a muntifunctional nucleus. (C) Benzoxazole (blue) as part of diverse natural products.

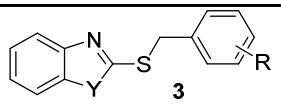
Table 1. Reactions of Benzyl Halides with **1a** and **1b**[a].



Entry	R	X	Product (Y = S)	Conversion[b]	Yields[c]	Product (Y = O)	Conversion[b]	Yields[c]
1	H	Cl	 **3aa**	97%	85%	 **3ba**	97%	75%
2	*p*-F	Cl	 **3ab**	99%[d]	75%	 **3bb**	91%[d]	74%
3	*p*-CF_3	Cl	 **3ac**	98%[d]	86%	 **3bc**	93%[d]	75%
4	*p*-CN	Br	 **3ad**	96%	79%	 **3bd**	94%	89%
5	*p*-NO_2	Br	 **3ae**	96%	82%	 **3be**	97%	77%
6	*o*-NO_2	Br	 **3af**	97%	72%	 **3bf**	98%	75%
7	*o*-Cl	Cl	 **3ag**	95%[d]	86%	 **3bg**	98%[d]	74%
8	*p*-Br	Br	 **3ah**	99%	79%	 **3bh**	98%[d]	89%
9	*o*-Br	Br	 **3ai**	96%	78%	 **3bi**	91%[d]	71%
10	*p*-I	Br	 **3aj**	97%	89%	 **3bj**	98%[d]	89%
11	*o*-I	Cl	 **3ak**	96%[d]	93%	 **3bk**	99%[d]	91%
12	*p*-CH_3	Cl	 **3al**	95%	91%	 **3bl**	98%[d]	88%

[a]**1a** or **1b** concentration was 0.30 M, **2a-l** concentration were 0.25 M. Reactions performed with 1 equivalent of KOH. [b]Conversions of **2a-l** by GC quantification. [c]Isolated yields. [d]New compounds.

Table 2. Antifungal Activity of Benzoazole Derivates **3**



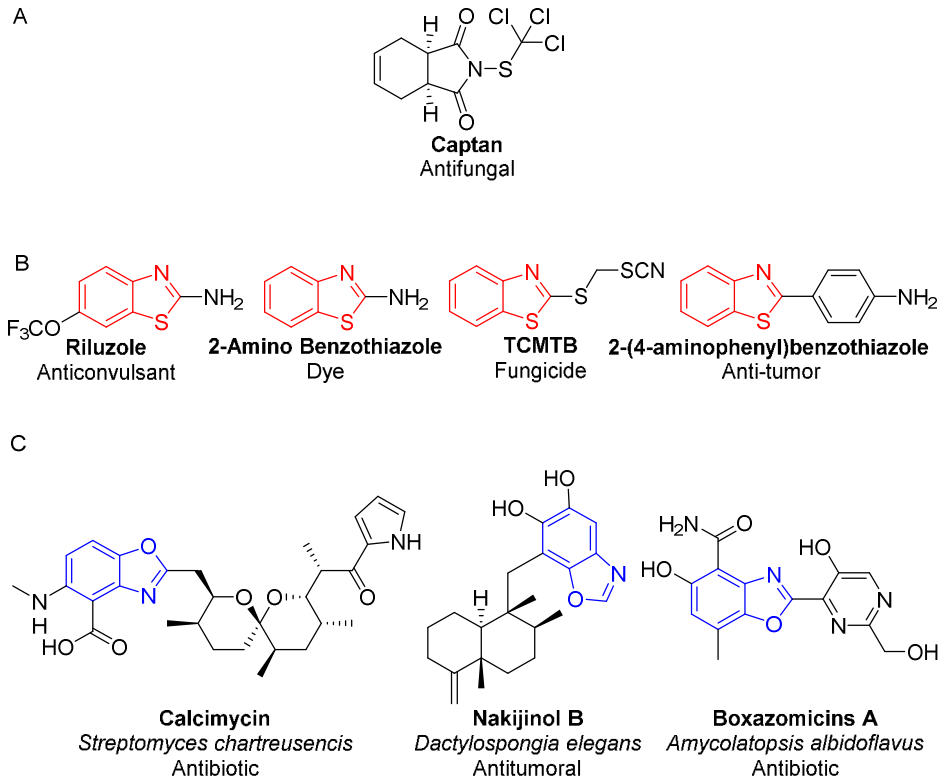
3

Compound	Y	R	Inhibition rate IC_{50} (μM)[a]					
			Botrytis cinerea	*Fusarium oxysporum*	*Aspergillus fumigatus*	*Aspergillus niger*	*Aspergillus terreus*	*Aspergillus ustus*
3aa	S	H	-	0.23 ± 0.05[b]	267 ± 40	5 ± 6[c]	13.2 ± 1.1	93 ± 10
3ab[d]	S	*p*-F	-	0.0067 ± 0.0005[e]	-	(6.5 ± 1.4)x10^{-39} [b]	56 ± 25	37 ± 6
3ac[d]	S	*p*-CF_3	10.3 ± 1.7	57 ± 5	-	34 ± 7	40 ± 6	0.0119 ± 0.0015[c]
3ad	S	*p*-CN	4 ± 7[f]	7.2 ± 1.2[g]	165 ± 12	-	22 ± 4	92 ± 12
3ae	S	*p*-NO_2	82 ± 13	29 ± 7	-	35 ± 11	-	-
3af	S	*o*-NO_2	149 ± 42	27 ± 5	46 ± 15	-	-	-
3ag[d]	S	*o*-Cl	1.1 ± 0.3[f]	27 ± 7	-	-	73 ± 18	229 ± 40
3ah	S	*p*-Br	8.7 ± 2.1	53 ± 8	-	-	127 ± 15	-
3ai	S	*o*-Br	40 ± 7	189 ± 10	161 ± 14	-	-	-
3aj	S	*p*-I	1.4 ± 0.9[c]	73 ± 3	-	-	112 ± 12	-
3ak[d]	S	*o*-I	39 ± 9	53 ± 11	39 ± 12	5.2 ± 2.3[b]	-	48 ± 8
3al	S	*p*-CH_3	20 ± 6	79 ± 7	29 ± 9	35 ± 11	-	62 ± 18
3ba	O	H	-	3.0 ± 2.0[f]	205 ± 16	60 ± 21	93 ± 7	173 ± 13
3bb[d]	O	*p*-F	26 ± 7	53.6 ± 1.9	104 ± 16	112 ± 19	108 ± 7	211 ± 40
3bc[d]	O	*p*-CF_3	36 ± 7	70 ± 7	5 ± 5[g]	-	44 ± 7	169 ± 27
3bd	O	*p*-CN	146 ± 30	111 ± 9	41 ± 11	61 ± 6	301 ± 30	38 ± 3
3be	O	*p*-NO_2	97 ± 13	141 ± 10	45 ± 4	18 ± 12	-	-
3bf	O	*o*-NO_2	-	63 ± 9	-	23 ± 4	-	-
3bg[d]	O	*o*-Cl	10.8 ± 1.9	46 ± 7	138 ± 20	-	102 ± 15	-
3bh[d]	O	*p*-Br	41 ± 7	45 ± 3	-	-	63 ± 9	-
3bi[d]	O	*o*-Br	1.4 ± 0.6[e]	29 ± 4	180 ± 29	-	196 ± 40	-
3bj[d]	O	*p*-I	57 ± 7	101 ± 11	46 ± 6	-	-	-
3bk[d]	O	*o*-I	33 ± 5	91 ± 9	22 ± 4	9 ± 4	-	46 ± 9
3bl[d]	O	*p*-CH_3	26 ± 7	139 ± 30	162 ± 8	10 ± 3	-	63 ± 16
Captan			17.8 ± 1.9	49 ± 13	15 ± 4	24 ± 6	55.8 ± 1.5	61 ± 4

[a](-): No activity (> 100 mg/L). [b]100% inhibition at 3.1 mg/L. [c]80% inhibition at 3.1 mg/L. [d]New compounds. [e]90% inhibition at 3.1 mg/L. [f]70% inhibition at 3.1 mg/L. [g]60% inhibition at 3.1 mg/L.

FIGURE GRAPHICS

Figure 1.



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